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Award Number: DAMD17-99-1-9068

TITLE: Development of a Novel Ligand Binding Assay for Estrogen Receptor

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REPORT DATE: April 2001

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20010620 217

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE April 2001	3. REPORT TYPE AND DATES COVERED Annual Summary (1 Apr 00 - 31 Mar 01)	
4. TITLE AND SUBTITLE Development of a Novel Ligand Binding Assay for Estrogen Receptor			5. FUNDING NUMBERS DAMD17-99-1-9068	
6. AUTHOR(S) Chi-Kong Arthur Chung, Ph.D.				
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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES This report contains colored photos				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) Nuclear receptors undergo conformational changes when they bind their cognate ligands. It should be possible to monitor these changes in vivo using resonance energy transfer between fluorphores. The existence of inherently fluorescent proteins such as the variants of jellyfish green fluorescent protein (GFP) suggests that this problem may be approached by making fusions of these peoteins to nuclear receptors. We set out to study this problem using the estrogen receptor (ER), a nuclear receptor known to undergo a conformational change upon ligand binding. We have proposed to generate a novel intrinsic ligand binding assay for the estrogen receptor based on ligand dependent conformational changes detected by fluorescence resonance energy transfer (FRET) between complimentary fluorescent proteins. We are in the process of cloning double and single chimeras of the estrogen receptor and the various fluorescent proteins into mammalian CMV expression vectors. We have extended the number of chimeras that we are generating because of the advent of new fluorescent proteins now available from Clontech, which include cyan, yellow and red fluorescent protein vectors. These new fluorescent proteins are more optimal for FRET than the original blue and green variants.				
14. SUBJECT TERMS Breast Cancer				15. NUMBER OF PAGES 8
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

FOREWORD

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Arthur Chung 4/27/01
PI - Signature Date

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Introduction

Nuclear receptors undergo conformational changes when they bind ligands. It should be possible to monitor these changes *in vivo* using energy transfer between fluorophores. The existence of inherently fluorescent proteins such as the variants of jellyfish green fluorescent protein (GFP) suggests that this problem may be approached by making fusions of these proteins to nuclear receptors. We set out to study this problem using the estrogen receptor (ER), a nuclear receptor known to undergo a conformational change upon ligand binding. The proposed assay we have set out to develop is shown in Fig. 1

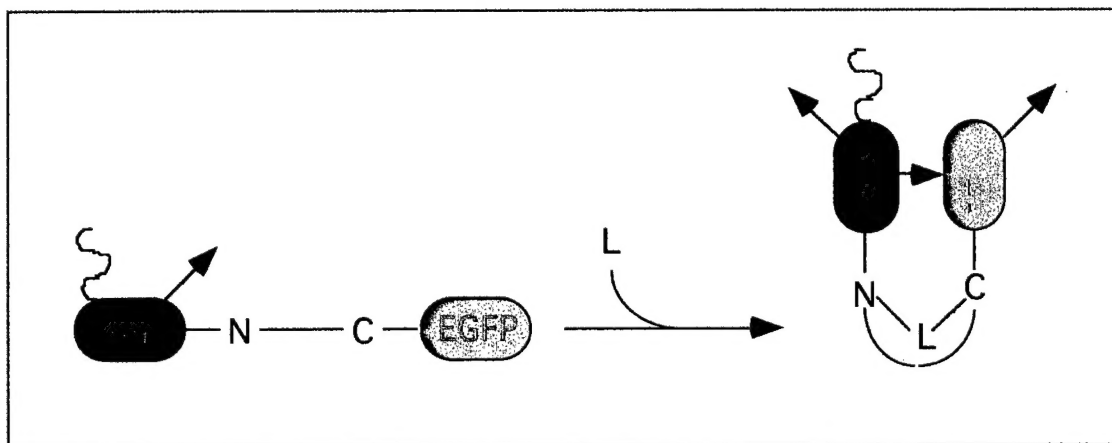


Figure 1: Ligand dependent steroid receptor assay based on FRET detection of conformational changes in the receptor upon hormone binding.

Summary of Progress 2001

Training:

I have gained much needed training in many areas of molecular biology including subcloning, protein expression, transfection of mammalian cell lines, and reporter assays. In addition, I am gaining biochemical training using hormone binding assays.

Technical Objective 1:

Task 1:

I initially proposed to create estrogen receptor (ER) chimeras with blue fluorescent protein (BFP) and green fluorescent protein (GFP) to generate a novel ligand binding assay based on fluorescence resonance energy transfer (FRET) between the two fluorescent reporters (Figure 1). In addition, we proposed last year to generate single and double receptor chimeras with cyan and yellow fluorescent proteins as well as receptor chimeras with the new coral red fluorescent protein. We have generated all of these receptor single and double fluorescent chimeras with complimentary fluorescent proteins. We have functionally tested all of these receptor chimeras in hormone binding and transcription assays. All of the jellyfish fluorescent protein receptor chimeras bind hormone with an affinities equivalent to that of wild type receptor. In addition all of these chimeras were able to transactivate, in a ligand dependent manner, reporter gene expression in transient transfection assays in HeLa cells. However, the transactivation levels were lower than that observed with wild type receptors, suggesting that the fluorescent protein moities may be disrupting the normal interactions of these receptors somewhat. However, these receptor chimeras were functional in that they bound ligand and activated gene expression. In contrast the red fluorescent protein receptor chimeras were inactive, both in hormone binding and transactivation assays. When we visualized these chimeras within the cells we observed they formed large inactive cytoplasmic aggregates. The red fluorescent protein receptor chimeras have to be re-engineered to alter the linker region to see if that will restore the functionality of the chimeras.

We went on to test the functional fluorescent protein receptor chimeras in FRET assays. We were unable to detect either ligand-dependent or ligand-independent FRET in transfected cells using confocal fluorescent microscopy. The fluorescent protein moieties may be disrupting the normal dimerization of the N-terminal domain with the ligand binding domain; thus the fluorescent protein partners would be too far apart to engage in FRET.

Task 2:

To be initiated.

Key Research Accomplishments:

Generation of receptor fluorescent protein single and double chimeras.

Reportable Outcomes:

None